ANGIOTENSIN II MEDIATED HYPERTENSION INDUCES LYMPHATIC CONTRACTILE DYSFUNCTION *EX-VIVO*

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Abstract

Hypertension (elevated blood pressure) is a risk factor for lymphedema after cancer treatment. Lymphedema is the fluid accumulation in the interstitial tissues due to impaired lymph flow. Lymph flow critically relies on spontaneous rhythmic contractions of lymph vessels (LVs) to maintain the interstitial fluid balance. Thus, evaluation of lymphatic contractions during hypertension is important to understanding the predisposition of cancer patients to lymphedema. Angiotensin II (Ang II) is a key regulator of blood pressure and potent vasoconstrictor. Ang II levels are elevated during hypertension, resulting in vasoconstriction, increased peripheral resistance, and increased afterload. However, the effect of Ang II mediated hypertension on lymphatic contractile function is unknown. Here, we evaluated how *in-vivo* Ang II administration modified the rhythmic contractions of isolated LVs *ex-vivo*. Ang II containing osmotic mini pumps (500 ng/kg/min) were implanted subcutaneously in male Sprague Dawley rats for 14 days. Isolated rat mesenteric LVs were cannulated and pressurized (4-5 mmHg) in a perfusion bath containing physiological salt solution (PSS). Real-time edge detection software tracked changes in diameter. Rhythmic contractions were recorded for ~45 minutes and then nifedipine (1 µmol/L) was added to the bath for 10 mins followed by washout with calcium free PSS. LVs isolated from Ang II infused rats (n=8) had significantly increased calcium dependent tone to 14.3% ± 5.1% compared to age-matched controls (n=8) which remained at 5.2% ± 1.1%. LVs from Ang II treated animals also exhibited a larger response to nifedipine (n=6) with 10.8% ± 5.6% increase in dilation compared to control LVs (n=8). Contractile traces showed visible dysrhythmia in LVs isolated from Ang II infused animals corresponding to a wider variation in time intervals between two consecutive contractions. Our findings suggest that Ang II mediated hypertension can lead to lymphatic contractile dysfunction, which may be a potential mechanism by which hypertension increases the risk of lymphedema.

Background

The lymphatic system moves fluid from the interstitial spaces to the bloodstream, with the help of lymph vessels (LVs) and maintains the fluid homeostasis. Rhythmic contraction is the main propelling force behind the transport of lymph fluid through the LVs, and any kind of LV contractile dysfunction can lead to fluid accumulation in the tissues, known as lymphedema. Hypertension is a risk factor which gives rise to lymphedema after cancer treatment; however, the effect of hypertension on LV contractility has not been fully defined. Studies have shown that Ang II mediated hypertension leads to increased Ca^{2+} tone and upregulation of L-type calcium channels (LTCCs) in arteries resulting in decreased arterial diameter. It is possible that hypertension results in similar changes to LV contractility. The purpose of our study was to determine the effect of Ang II mediated hypertension on LV diameter and rhythmic contractions of rat mesenteric LVs.

Methods

Rats were placed in an induction chamber with an inflow of isoflurane at a rate of 1.5-2%. An ~1 cm incision was created behind the ear over the shoulder blade of the front leg to make a subcutaneous tunnel. Ang II containing osmotic mini pumps (500 ng/kg/min) were then implanted subcutaneously in 9-13 weeks old male Sprague Dawley (SD) rats for 14 days. Second order mesenteric LVs were isolated and cannulated from Ang II treated and age matched control rats. Vessels were pressurized to 4-5 mmHg and allowed to equilibrate in physiological saline solution (PSS) at 37°C to allow spontaneous contractions to develop. Edge-detection software (IonOptix) recorded changes in external diameter. Spontaneous contractions were recorded for ~45 mins followed by washout with calcium free PSS for 10 mins. In a subset of LVs, nifedipine (NIF; 1 µmol/L) was added to the bath prior to washout with calcium free PSS.

Methods

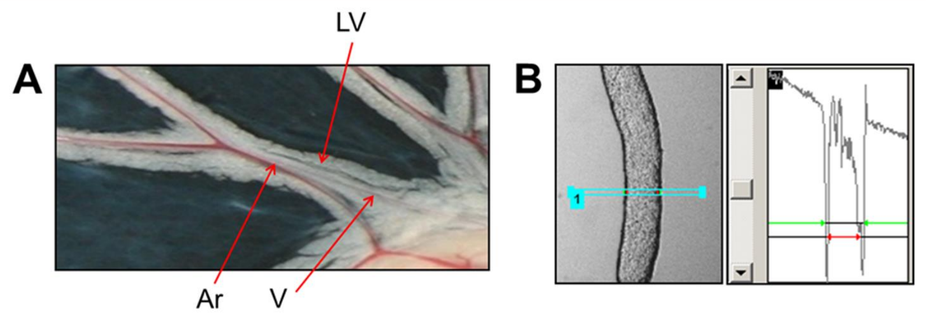

Figure 1: ***(A)*** Lymph vessel (LV), artery (Ar) and vein (V) in a rat mesenteric loop. ***(B)*** Image of a cannulated LV; diameter is measured by edge detection software.

Results

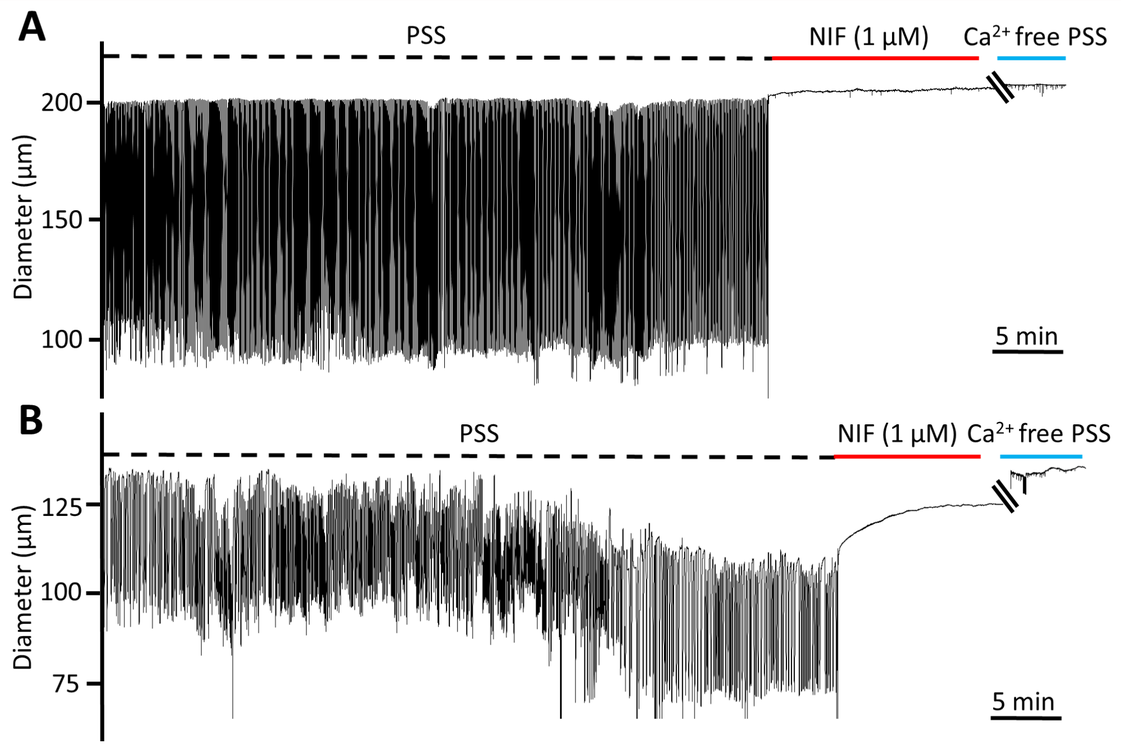

Figure 2: Effect of *in vivo* Ang II administration on LV contractions *ex vivo*. Representative LV diameter traces for ***(A)*** control rat and ***(B)*** Ang II treated rat.

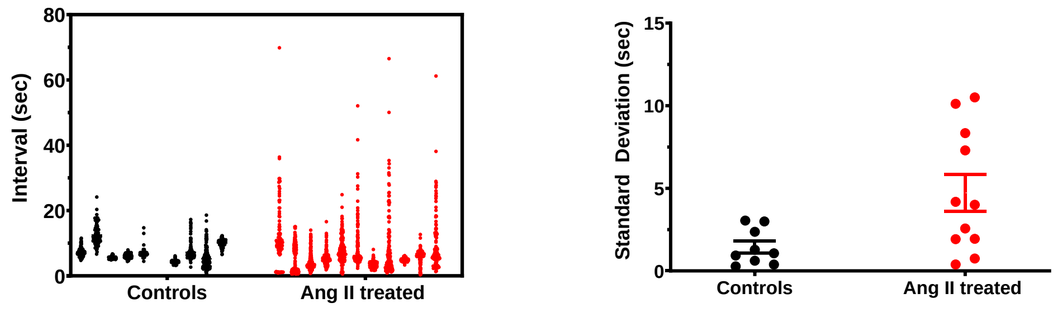

Figure 3: Dysrhythmic contractile pattern of LV corresponds to a more varied contraction interval. ***(A)*** Manhattan plot showing each interval between two consecutive contractions in individual LVs. ***(B)*** LVs isolated from Ang II treated rats exhibited increased variation in the standard deviation for contraction interval compared to controls (n= 9-11).

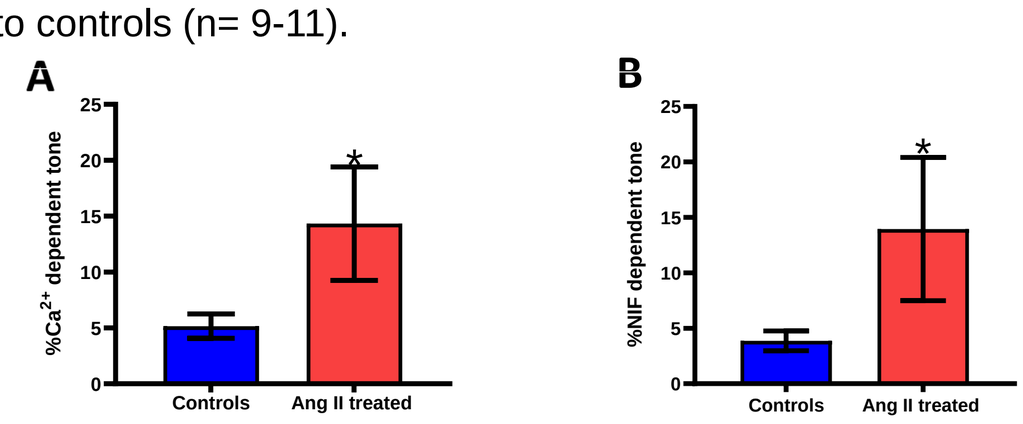

Figure 4: Ang II treated LVs exhibited increased Ca^{2+} dependent tone. LVs isolated from Ang II treated rats had increased dilation in response to calcium free PSS and nifedipine corresponding to an increase in ***(A)*** global Ca^{2+} dependent tone and ***(B)*** tone mediated by LTCCs. Data presented as mean ± SEM (n=6-8; $p<0.05$)

Summary

- **Ang II mediated hypertension induced contractile dysrhythmia in isolated LVs.**
- **LVs isolated from Ang II treated rats exhibited increased calcium dependent tone.**

Future studies are needed to determine the molecular mechanism underlying this lymphatic contractile dysfunction and whether Ang II mediated hypertension alters lymph flow in vivo.

Acknowledgments

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